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MITOSIS IN SYNCHYTRIUM

WITH SOME OBSERVATIONS ON THE INDIVIDUALITY OF THE CHROMOSOMES[1]

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(WITH PLATES XVI–XVIII)

The nuclei of *Synchytrium decipiens*, a leaf parasite of the hog peanut, were shown in previous papers to be derived very largely by amitosis. Several sorts of amitosis were observed, two of which, nuclear gemmation and heteroschizis, differ considerably from the ordinary process of amitosis. The nuclei derived by these direct divisions were shown to be persistent, and some of them were observed to divide by mitosis. This with other facts led me to the conclusion "that the nuclei derived by these processes of amitosis are normal, and that they with their descendants become the functional nuclei of later generations, capable of perpetuating the species" (**5**, p. 134). The purpose of the present paper is to describe the mitoses which follow, to correlate them with the amitoses, and to discuss the theoretical bearing of the facts thus presented.

I would here repeat my acknowledgments to my friend, Professor F. L. STEVENS, of the North Carolina College of Agriculture and Mechanic Arts, who furnished the material used in the investigation and gave the suggestions that originally aroused my interest in the problem. The sections were cut 2–10 μ thick and stained with Haidenhain's iron alum hematoxylin and with anilin safranin and gentian violet.

As in coenocytes generally, all the nuclei in a cyst pass into mitosis simultaneously and divide with equal rapidity, so that all of them are very nearly in the same stage throughout. This fact may be utilized for overcoming one of the most serious obstacles encountered in investigating the cytology of this plant—the difficulty of estimating the relative ages of the different structures in the absence of any

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[1] Contribution from the Botanical Laboratory of the Ohio State University, No. XLIX.

indications external to the nuclei themselves—for those cysts which are of critical age usually contain short series of closely connected stages which frequently enable one to assure himself concerning difficult points, such as the formation of the spindle or the origin of the asters. Nuclei of widely different phase in the same cyst are very rare: *fig. 5* is a case where the small nuclei in the cyst were still in the vegetative condition while the large nuclei had passed into spirem; *fig. 35* shows a case where the small nuclei had reached late anaphase while the large nuclei were still in metaphase; *fig. 36* of the previous paper (**5**) is a case where the reverse was true, the small nuclei being in metaphase while the large ones were in anaphase. It would be a matter of great interest to ascertain the mechanism by which the nuclei are kept in phase. While not throwing much light on the nature of the stimulus, the behavior of newly segmented cysts is interesting in this connection. In such a cyst, long before the walls of the zoosporangia appear, while the segments are still separated from each other only by an exceedingly delicate plasmatic membrane, each nucleus has become entirely independent of the others; and one finds vegetative nuclei, metaphases, and telophases in adjacent segments, showing how slight a separation suffices to establish the complete physiological autonomy of the individual nucleus (*fig. 33*).

All of the mitoses of Synchytrium are of the same type. It is true that none of the later nuclei undergo such a shrinkage in volume with its associated peculiarities as occurs preparatory to the primary mitosis (STEVENS **18**), but these phenomena are probably due simply to the enormous size of this overgrown nucleus. It may be recalled that similar peculiarities are usually observed in very large nuclei wherever they are found. CHAMBERLAIN (**1**), for example, was not able to interpret the structures he found in the enormous egg nucleus of Dioon. But aside from these peculiarities of the primary nucleus, the only differences that could be detected were in the size of the spindles, those in the early stages being fairly large, while those in the last mitoses of the sporangium are exceedingly minute (*figs. 32–34*).

In the resting nuclei of Synchytrium the chromatin is all concentrated in a single globular karyosome (*figs. 1, 31*). Except in those cases where vacuoles have appeared on the removal of the chromatin preparatory to mitosis or nuclear gemmation (*fig. 36*), the karyosome is an entirely irresolvable, deeply colored body, whether stained with hematoxylin or safranin. Besides the karyosome there may be a few deeply staining granules on the nuclear membrane, but there is nothing corresponding to the chromatin reticulum characteristic of the vegetative nuclei of many cells. The only nuclei in which the chromatin approached the condition of a reticulum were located in the degenerating cysts occasionally found.

The spirem

The early prophases of mitosis consist in the formation of the spirem from this compact karyosome. This is brought about in the most direct manner possible. The karyosome first separates into a number of irregular chromatin masses (*figs. 2, 30*); next delicate linin bands appear connecting these granules with the nuclear membrane (*fig. 3*); and along these bands the chromatin granules are distributed over the nuclear cavity, forming the expanded spirem (*fig. 4* from the same cyst as *fig. 2*).

Only a portion of the spirems of Synchytrium, however, are destined to pass into mitosis. A large proportion of them undergo nuclear gemmation or some other form of amitosis. In view of theoretical considerations which will be discussed later, it is of the utmost importance to determine whether the spirems of amitosis are of the same nature as those of mitosis, or whether they are different in kind and present only accidentally an optical similarity. In most cases it is easy to distinguish the two sorts by the cysts in which they occur. In those cysts which are in the prophases of mitosis, the nuclei are in general very nearly the same size and evenly spaced off from each other, while in the amitotic cysts there are small nuclei in groups or clusters as well as the large spirems. Many such spirems have a strikingly different aspect from the mitotic spirems. They are coarser (*fig. 37*), the chromatin granules are very much larger, and the linin strands are short and heavy; whereas the mitotic spirems are characterized by linin threads and chromatin granules of various sizes lying side by side. In the amitotic spirems each of the chromatin granules is evidently simply the karyosome of a small nucleus, for the formation of which the spirem is a preparation. It would thus appear that the two classes of spirems are entirely different from each other, but unfortunately for this view a very large proportion of the amitotic spirems are intermediate, or so closely resemble the mitotic spirems that they can be distinguished from them only by the character of the cyst in which they occur (*fig. 41, a, b, c*). It seems impossible therefore to determine certainly whether these two classes are distinct or whether they are of the same nature.

The origin of the spindle

In those cells where the spindle is intranuclear, there seem to be two types of spindle formation. In the first type, which occurs in the ascomycetes (e. g., Phyllactinia, HARPER **8**) and brown algae (e. g., Fucus, YAMANOUCHI **21**), the centrosomes are permanent organs of the cell and form the spindle by moving around the nucleus so as to include the chromosomes between them, organizing the spindle by the union of those astral rays which penetrate the nucleus in a manner very similar to that prevalent among animals. This process is very conspicuous and has been observed and figured by numerous investigators. In the other type, which occurs in the oomycetes (e. g., Saprolegnia, DAVIS **3**; and Albugo, STEVENS **16**), centrosomes are either small or absent in the early stages; to this type the spindles of Synchytrium belong. Here the determination of the origin of the spindle is very difficult. This probably accounts for the fact that very few of those who have figured such mitoses have given a series of figures of the prophases complete enough to throw much light on the formation of the spindle.

When first seen, the spindle of Synchytrium, which is thrown directly across the cavity, is not distinguishable by its staining reaction or otherwise from a strand of the spirem (*figs. 6–8*). It is therefore difficult to be certain just when the spindle appears or what it comes from, but it gives every indication of being differentiated from a spirem strand. Very soon, however, it becomes sharply pointed and quite different from the spirem, which now begins to be drawn in around its equator (*figs. 9, 10*). Contraction continues and more definite connections between the chromatin and the spindle fibers are established (*figs. 11, 12*). In this condition the spindle often appears bipolar (*fig. 9*), which emphasizes the similarity of the linin strands and the spindle fibers. By further contraction this much shortened spirem is converted into the four chromosomes of metaphase (*figs. 14, 15*). Very frequently, however, the contraction is so pronounced that the individual chromosomes cannot be made out in the chromatic mass at the equator (*fig. 13*).

Some spindles show nucleoli lying in the nuclear cavity beside the spindle; in others no nucleolus is present. No difference was observed between the spindles with nucleoli and those without; but it was observed that in any given cyst all of the spindles were alike in this respect—either all had nucleoli or all lacked them. In rare cases more than one of these bodies were present. The nucleoli remain beside the spindle till after the daughter nuclei have separated in telophase (*figs. 22, 29, 33*), when they disappear.

As soon as the spindle is formed, the nuclear membrane with the small chromatic granules which are imbedded in it begins to disappear (*figs. 10–15*), soon leaving the spindle free in the cytoplasm. As is usual with the intranuclear spindles of fungi, the metaphase in which the chromatin is all concentrated at the equator, if we may judge from the frequency with which it is observed, is of long duration. This is in contrast with the mitoses of the higher plants, where good metaphases, far from being the commonest, are observed less frequently than other stages.

Anaphase and telophase

The spherical chromosomes are pulled away from each other in the usual manner by the fibers of the spindle, which are exceedingly heavy (*figs. 15, 17*). In early anaphase, figures stained with anilin safranin and gentian violet show the chromosomes red and the spindle violet but in hematoxylin preparations the chromosomes are difficult to differentiate from the spindle fibers. This difficulty increases in late anaphase until it becomes impossible to distinguish chromatic from achromatic structures, even when stained with the safranin-violet combination. When the chromosomes pull apart, they remain connected by heavy fibers similar to those by which they were pulled away from each other (*fig. 18*), which persist long after the chromosomes become lost in the condensed mass at the poles (*fig. 19*). These deeply staining fibers are very conspicuous and give a characteristic appearance to side views of anaphases, which somewhat resembles the daughter stars formed in mitoses where the chromosomes themselves are elongated. These fibers may be used to determine the chromosome number, since each one of them is connected with a chromosome. This can be done more easily in this stage than in metaphase, because the fibers are larger than the chromosomes and because they spread apart as they separate, sometimes giving views of all four at once, while in any other stage the full number can be seen only from polar view (*fig. 16*).

These radiating bands of fibers are now drawn in, leaving the two poles connected only by a central band (*figs. 20, 22*), which becomes considerably attenuated as the two chromatic masses move farther and farther apart. The daughter nuclei become separated by distances considerably greater than the original length of the spindle. In moving apart one or both of them frequently swerves from the axis of the spindle, so that the connecting fibers meet at a considerable angle (*fig. 33*). As was indicated in the paper on the reconstruction of the nucleus (GRIGGS 4), this habit makes it almost impossible to associate the daughter nuclei in pairs after the wisps of spindle which point to the original position of the equator have disappeared, especially since the wide separation greatly lessens the chances of securing both in the same section. For this reason all figures of stages after this period are drawn from only one of the daughter nuclei. After the spindles have broken in two, the telophases are very inconspicuous objects; though they stain deeply, the chromatin is concentrated into so narrow a space that they are practically invisible under any but the highest power (*figs. 23, 35b*).

The aster

As previously reported by STEVENS, KUSANO, and myself, there are no granules, radiations, condensations, or any other indications of the presence of centrosomes at the poles of the spindle during prophase or metaphase. But about the time the two halves of the spindle separate, radiations begin to emanate from the chromatic masses. At first so delicate as to be on the very limit of visibility, the aster rapidly increases in prominence until it becomes exceedingly conspicuous, clearly visible under a magnification of forty or fifty diameters. At first the radiations may appear to emanate from the center of the chromatic mass (*figs. 24, 25*), but very soon it is evident that the focus is beyond the condensed chromosomes (*fig. 24*). As the rays increase in strength the focus is shifted, until there is a considerable interval between the center and the chromatin (*fig. 27*). In this stage, since the chromatin is condensed to its minimum volume, the aster is so very much more prominent than the chromatin that the latter is likely to be overlooked altogether, making it appear that the cyst has no nuclei, but only asters! The chromatic mass soon enlarges, however, and rounds off into the spherical karyosome of the resting nucleus (*figs. 27, 28*). Up to this stage the chromatin lies suspended in the cytoreticulum, without any apparent relation to it. The appearance of the karyosome, however, marks the resumption of definite relations of nucleus and cytoplasm in the formation of a vacuole around the chromatin (*fig. 28*). This vacuole is at first bounded only by the meshes of the cytoplasm, but soon the rays of the aster bend around it and form the heavy nuclear membrane which incloses it (*figs. 29, 30*), as previously described by KUSANO (**10**) and myself (**4**). When the nuclear membrane is complete, the aster gradually disappears; the rays first become much more numerous and finer; the center gradually becomes diffuse and stains less deeply (*fig. 4*); and finally the aster is transformed into a condensation of cytoplasm as previously described (*figs. 31, 20, 3*).

During the disappearance of the aster, however, the nucleus usually enters into the prophases of the next division, so that only seldom is a cyst found where the nuclei are still in their vegetative condition when the asters are in their last stages. On the other hand, it is not at all unusual to find that the new spindle has already formed, before the aster has disappeared, and in rare cases the anaphase of the succeeding division (*fig. 20*) may be reached before the old aster is completely gone. The disappearance of the aster probably occupies a period of somewhat definite length, while the rapidity with which the mitoses succeed each other varies greatly in different cysts. It was this lack of correspondence between the cycles of astral and nuclear metamorphoses that made it necessary in the former paper on this subject to state some conclusions provisionally that may now be positively established.

From their function of forming the nuclear membrane, the asters of Synchytrium have been named *karyodermatoplasts* by KUSANO (**13**). Although somewhat long, this term may be useful in alluding to the *function* of the asters, but there seem to be certain objections to its use as the name of a structure. First, the structure so designated is so variable that it is difficult to define it. Sometimes the aster is single, sometimes double or triple (*fig. 2*); sometimes it has one clearly defined granule at the focus of the rays; more often there are several such granules more or less eccentrically placed; or there may be none at all. Sometimes there is an elongated band which bears radiations all along its length, like the blepharoplast of a cycad. It is evident that the term karyodermatoplast can be defined only by its function, while such a term should rest on a morphological basis. Second, though the aster, so far as observation has yet indicated, serves only to reconstruct the nuclear membrane, that function alone does not seem to the writer adequate to account for its enormous development in Synchytrium. This is more apparent when one recalls the fact that in heteroschizis and nuclear gemmation the membranes of the daughter nuclei are formed without the intervention of any such structure. I have preferred, therefore, in the present discussion to employ the descriptive term aster for the structure in question, without committing myself to its significance. The question of the radiate structures in Synchytrium and their homology is one of very great interest and deserves consideration in a separate paper.

The chromosome number

Inasmuch as most of the nuclei or their ancestors have been derived by amitosis, as has been previously shown, the determination of the number of chromosomes in Synchytrium becomes a matter of much more importance than in organisms where the orderly sequence of mitosis has not been interrupted. On this account especial care has been taken to insure accuracy of observation and interpretation. Upward of 500 slides have been used in the study. Altogether several hundred mitotic cysts with many thousands of individual spindles have been observed. Of these only the most favorable were used for basing the conclusions. The location of the cysts containing these most favorable spindles was recorded by vernier readings of the mechanical stage and filed in a card catalogue, so that they could be reexamined in rapid succession as frequently as desired. The favorable cysts so listed number about fifty; most of them contain several hundred spindles.

All of the results obtained were not exactly concordant, but I shall give the observations on which the conclusions were based that the reader may be enabled to judge of their soundness for himself. In all but two of these favorable cysts there were constantly four chromosomes. In these two, however, the number of chromatin bodies was certainly more than four (*fig. 42*). But while the chromosomes are all of the same size, some of these bodies were smaller than chromosomes and had the appearance of masses of chromatin from the late spirem which had not yet fused together into the compact chromosomes of metaphase. Inasmuch as the spindles were undoubtedly newly formed, this is probably the correct interpretation, especially as there seemed to be in some cases faint wisps of spirem remaining about the equator of the spindle. But whether these supernumerary chromatin bodies are to be explained on some such basis or whether they are actual irregularities in the number of chromosomes, I am of the opinion that they do not seriously weaken the conclusion that the chromosome number is four. Although it is unusual for a writer to state difficulties of this sort as frankly as has WILSON (**20**) in his study of Metapodius, seeming discrepancies in the number arising from one cause or another are, I believe, frequently met with in efforts to count chromosomes.

In all of the other cysts studied there was no deviation from the constant number four. On all of the spindles the chromosomes were placed at angles of about 90°, so that two, three, or four of them were visible, according to the angle of observation (*figs. 14–19*). In the very much larger number of cysts whose spindles were not favorable enough to permit exact counting, there were no indications of a different number. It should be noted also that four is an exceedingly easy number to count, for one can *see* four without counting them one by one as is necessary for a larger number. In thus maintaining a constant number of chromosomes the writer is supported by the only other published accounts of the chromosomes of Synchytrium. STEVENS in his paper on the primary mitosis (**18**) states (p. 413) that "they are probably four in number, although we do not assert this with certainty," and in his second paper he shows spindles with the same number of chromosomes. KUSANO (**10**) reports definitely five chromosomes in *S. puerariae*.

The probability that the results thus obtained are accurate is very greatly increased by the fact that in Synchytrium there is no differentiation into soma and germ plasm. Every nucleus becomes either directly or through its descendants the nucleus of a spore, which, if successful in entering its host, becomes the large nucleus of a primary cyst. If a variation of the chromosome number occurred at any point, it would, on the individuality hypothesis, be perpetuated indefinitely, affecting all the nuclei of later generations; whereas in the higher plants and animals a variation in the somatic nuclei would be lost with the death of the individual organism in which it occurred. It is clear, therefore, that if a variation in the number of chromosomes should occur only once in the repeated direct divisions through which the nuclei of the spores have been derived, it would affect all the nuclei of the next generation. Thus, though the nuclei of the later stages of the parasite are so small that an irregularity in the chromosome number might not be detected, yet in the large nuclei of the succeeding generation it could not escape observation. Amitosis plays so important a part in the formation of the nuclei that if chromosome variations occurred in only one per cent. of the direct divisions, the nuclei of the whole parasite would in the course of a few generations become so irregular that it would be impossible to recognize the original number of chromosomes when it did occur. It may also be remarked that an irregular reduction in the number of chromosomes, such as might be expected in the amitoses of a non-sexual organism like Synchytrium, could lead, when repeated in indefinite series, to only one result—all the nuclei would finally have only one chromosome.

On the individuality of the chromosomes

Modern cytology may be almost said to be built around the theory of the individuality of the chromosomes, and certainly no other hypothesis has borne so much fruit in valuable results as this. Without it the complicated process of mitosis would seem to lack significance, and the doubling and reducing of the chromosomes in fertilization and reduction would be well-nigh meaningless. For its support it has had, besides the constancy of the chromosomes in the species, various observations on nuclei which were known to contain either more or less than the normal number of chromosomes.

The great difficulty in the way of the theory has always been that the chromosomes apparently lose their individual existence during the vegetative phases of the nucleus. In many cases where the nuclei are rapidly dividing the chromosomes do not, however, entirely lose their individuality between the successive divisions, but divide and reproduce themselves directly from the compact condition of mitosis. These cases have been the evidence by which the vegetative period of the nucleus was bridged over by the individuality hypothesis, for there is an unbroken series of intergradations from this condition to that in which the chromosomes have completely lost their morphological identity, and one would not suppose that nuclei in one condition differed fundamentally from those in the other. Accordingly, basing their statements on observations of nuclei in which the individual chromosomes could be traced, with more or less certainty, from mitosis to mitosis, several writers have asserted confidently that the individuality of the chromosomes is maintained through all conditions of the chromatin, whether visible or not.

But the transformations of a set of chromosomes which, after they are once formed, persist and divide are not necessarily equivalent to the formation of a new set from a diffuse reticulum. If the chromatin reticulum gave rise only to the chromosomes, the analogy might be closer, but when, as frequently happens, large amounts of chromatin are cast out into the cytoplasm as nucleoli, microsomes, or in mass, it is evident that the succeeding differentiation of the chromosomes involves factors different from those entering into the mere transformation of a chromosome which persists *intact* through whatever metamorphoses it may pass. This may be illustrated perhaps by comparing the chromosomes to metal rods which remain distinct from each other when cold, but as the temperature approaches the melting point become soft, lose their shape, and partially fuse together, though each retains its distinctness and can be reclaimed unchanged on cooling. But after they have once melted together, all this is changed, and they can be regained from the homogeneous flux only by making them entirely over again. It seems quite possible that in the diffuse vegetative reticulum the chromosomes are as completely fused as the molten metal, and until we know more than we do now concerning the nature of the reticulum, this alternative possibility should be kept before us. The analogy of the molten metal is more suggestive in cases like Synchytrium, where the chromatin is partially or completely concentrated in a karyosome from which part or all of the spirem is directly derived. Although cytologists have as a rule paid but little attention to their behavior in mitosis (cf. WAGER **19**), such structures are common occurrences in many plants and animals.

If we may infer safely, as I believe we may, that the chromosomes of *Synchytrium decipiens* number constantly four, it becomes a matter of primary interest to determine how that constant number is maintained in all of the amitoses through which the nuclei pass. It has long been assumed that the principal if not indeed the sole function of the complicated process of mitosis was to insure exactly equal division of the chromatin between the daughter nuclei. In view of the fact, however, that the majority of the nuclei of Synchytrium are derived by some form of amitosis either directly or through their ancestors, and yet maintain the number of their chromosomes constant, it is evident that mitosis is not necessary to maintain this number.

The possibilities of an exact mechanical division of the chromatin are somewhat different in the different varieties of amitosis. In heteroschizis there occurs a metamorphosis of the nucleus suggestive of that of mitosis. In the loss of the nuclear membrane and the apparent cessation of interaction between the nucleus and the cytoplasm there may be a pause during which the chromatin is divided granule by granule in such a way that the karyosomes of the daughter nuclei are furnished with exactly equal chromatin content, just as is visibly accomplished by the fission of the chromatin granules or the chromosomes in mitosis.

Likewise it is not inconceivable that the karyosome may be divided in a similar manner when it is broken up preparatory to nuclear gemmation, though in this variety of amitosis there is no loss of the nuclear membrane or other indication of a pause in metabolism.

An exact equational division of the chromatin under such circumstances would be something of a novelty, but it would not necessarily involve functions of the chromatin differing fundamentally from some with which we are more familiar. Whenever the chromatin is withdrawn granule by granule from a karyosome (nucleolus) preparatory to mitosis, we must suppose, on the individuality hypothesis, that the granules come out of the nucleus as they went in, properly sorted, so that each granule is taken into that chromosome from which it came. On the assumption of an equational division in heteroschizis and nuclear gemmation, we should have to assume, in addition to this well-known property of the chromatin granules to sort themselves out from the apparently homogeneous mass of chromatin of the karyosome, only the further property of reproducing themselves while yet within the karyosome as they ordinarily do in the spirem. This is perhaps not too much to ascribe to the chromatin, but even such an addition to our theory would add a very large field for speculation where the conclusions, with our present methods, could never be checked by observation.

But when nuclear gemmation takes place in spirem two alternative possibilities are presented, the choice between which depends on the nature of the spirem involved. If the spirem is an entirely different structure from the mitotic spirem, it would be rational to suppose that each small karyosome, i. e., each granule of the spirem in nuclei such as *fig. 37*, contains four chromosomes derived by an equational division of the mother karyosome as in the previous cases. But if the amitotic spirem is like the mitotic, the situation is entirely different, for we know from its later history the composition of the mitotic spirem. It contains altogether only four chromosomes, which are arranged serially, and any small part will contain chromatin from only *one* chromosome, if a small part of such a spirem is extruded and forms an independent nucleus. Therefore, it is evident that its chromatin is derived not from all four but from only one chromosome. It is then a matter of great importance to determine the constitution of the spirem, but unfortunately, as stated above, the evidence on this point is somewhat ambiguous, and a definite decision in favor of either alternative seems impossible.

Certain features of nuclei dividing by constriction, however, seem to throw some light on this matter. When amitosis by constriction occurs, the nucleus develops lobes and divides up into a number of daughter nuclei varying from two to a dozen or even more (*fig. 41, a, c*). One cannot tell by observation whether a nucleus is about to divide into two or many daughters, for there is no apparent segregation of chromatin into parts corresponding in number with the number of small nuclei to be formed. Nor are the small nuclei necessarily equal in size or chromatin content. Sometimes the variations are very great; thus *fig. 36* shows a cyst in which the primary nucleus has divided, leaving the nucleolus undivided in one of the daughters, while the other and smaller daughter nucleus has constricted or budded again to form a relatively minute nucleus at one side. It is difficult to believe that these three nuclei had their origin in equational divisions of the chromatin previous to the actual constriction of the mother nuclei. All appearances go to indicate rather that the factors controlling the division of these nuclei are entirely disconnected from the behavior of the chromatin, and favor CHILD's hypothesis (2) that amitosis is merely a physical process. But whether this be the case or not, nuclei so formed are normal and sometimes pass into mitosis. When this happens they show the four chromosomes characteristic of the species (*fig. 35, a*). More commonly, however, amitosis by constriction is but an incident in the division of the chromatin, for the constricted nuclei are soon completely converted into groups of small nuclei by gemmation (see **6**, *fig. 3*). Since the constitution of these nuclei is of great importance in this connection, I have introduced here a series of three drawings of nuclei from the same cyst. *Fig. 38* shows a case of amitosis of the type most commonly observed, though it is not common in Synchytrium; in *fig. 39* the two nuclei are completely separated but still touch each other; in *fig. 40* one of the granules similar to those on the periphery of the other nuclei has formed a small nucleus by gemmation. The individuality hypothesis leads us to rather startling conclusions regarding the constitution of these nuclei. Each of the large nuclei must have four chromosomes. And since each of the granules on the periphery has the power of organizing a new small nucleus with four chromosomes, it also, if the chromosomes have a material continuity from generation to generation, must contain four chromosomes. In other words, the large nuclei must at one and the same time have four and $n \times 4$ chromosomes. In these nuclei the small karyosomes number 4, 5, 6, 7. The respective nuclei must therefore have 16, 20, 24, 28 chromosomes, and still have no more than four!

There seems to be no option but to conclude that in Synchytrium there is no morphological continuity of the individual chromosomes, nor is there any definite set of chromatin granules of whatever size which are passed on intact from nucleus to nucleus. The number of chromosomes seems to be a physiological rather than a morphological constant. Not the chromosome but the nucleus itself seems to be the morphological unit of the lowest order. Apparently any mass of chromatin that is capable of organizing a nucleus at all, i. e., any particle of nuclear matter that is able to continue life and reproduce itself, whether it originally contained portions of all of the chromosomes or of only one of them, will preserve the characteristic number of chromosomes along with the other hereditary characters of the species.

Although such a hypothesis seems to be required to explain the phenomena of nuclear division in Synchytrium, it would not be wise to attempt to reach any decision as to its general applicability at this time. In proposing it to account for these features of Synchytrium, the writer is not at all oblivious of the enormous amount of evidence which has been heaped up in recent years in support of the individuality of the chromosomes. The occurrence of protochromosomes in the nuclei of many cells (OVERTON **14**); the persistence of supernumerary chromosomes in cases of polyspermy, etc.; and the remarkable size and shape differences among the individual chromosomes such as have been discovered in animals, notably by McCLUNG and MONTGOMERY, and recently in plants by SCHAFFNER (**15**) and Miss HYDE (**9**), along with many other observations of a similar nature, are facts which give very great weight to the hypothesis of the individuality of the chromosomes. Nevertheless, it must be remembered that almost all of the evidence in favor of the individuality hypothesis is of suggestive rather than demonstrative value. This was clearly recognized by all observers until about five years ago, but since that time the amount of such evidence has increased to such an extent that cytologists have been much less cautious than before in using the hypothesis. The renaissance of MENDEL'S law so favored the general acceptance of the chromosome hypothesis by making it highly desirable on theoretical grounds to find the material bearers of hereditary units which the law seemed to demand, that practically all opposition to it was swept away, and it has come to be the groundwork on which present-day cytology has been constructed. If then the basis on which the theory rests is in any degree unproven, it is well for cytologists to keep that fact clearly before them, that they may be guarded against making any false steps in blind adherence to an insecure hypothesis or of failing to notice any evidence which might throw doubt on the validity of their theory.

The writer for his own part, however, has no intention of discarding the whole hypothesis of chromosome individuality on the basis of these observations on Synchytrium, or on the similar results obtained by CHILD (2) from studies of representatives of nearly all the great animal phyla. The conflict of evidence for and against the hypothesis seems clearly to require us to keep the case open for the present. It is as tending to promote such a judicial attitude of suspended judgment among cytologists rather than as overthrowing the whole theory that the writer would have his results received.

Summary

The mitoses are all of the same type and occur simultaneously throughout the cyst.

The spirems of amitosis are frequently indistinguishable from those of mitosis.

The spindle is of the oomycete type, without centrosomes.

The asters first appear as emanations from the condensed chromatic mass of telophase, but quickly separate from the chromatin and their rays form the nuclear membrane.

The number of chromosomes is constantly four.

In some sorts of amitosis an equational division of the chromatin previous to the division of the nucleus is possible, though there is no evidence of such a process.

In other varieties of amitosis an equational division of the chromatin does not seem to be possible, but rather direct division gives some indication of being merely a physical process as suggested by CHILD.

Nevertheless, nuclei known to be derived by amitosis show four chromosomes.

It is therefore concluded that in Synchytrium there is no morphological or material continuity of the chromosomes from generation to generation of nuclei; but that the chromosome number is a physiological constant, like the other hereditary characters of the species.

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Addendum

After the foregoing paper had been completed and submitted for publication, KUSANO'S "Contribution to the cytology of Synchytrium and its hosts" (Bull. Col. Agr. Imp. Univ. Toyko **8**:80–147. *pls. 8–11.* 1909) reached me. KUSANO'S paper, for the most part, is based on observations of *S. puerariae*, which seems to be remarkably similar to *S. decipiens*, which he also used for comparison. He gives much space to the metamorphosis of the nucleolus (karyosome), which is unusually favorable for study in Synchytrium, showing that all the elements derived from the mother nucleus are concentrated in the karyosome of the daughter nucleus from which they are later withdrawn. He figures the prophases and metaphases of the primary mitosis, confirming for the most part STEVENS' observations, but like him failing to find the anaphases and telophases, which for some reason seem to be very difficult to observe. He then devotes considerable space to the secondary mitoses, obtaining results similar to those of the present paper; but his account of the prophases differs considerably from that of the present writer. His series of figures, however, is not complete at this stage, and he admits (p. 102) that he had not been able to follow the formation of either the chromosomes or the spindle. Again, in the telophase there is a wide gap between his *figs. 55* and *56*, during which the aster is developed, a process about which he was left to conjecture (p. 127), incorrectly supposing that it originates from the cytoplasm. In addition to the method of segmentation by cleavage furrows described by HARPER, he reports a second method in which the sporangium walls are precipitated by the cytoplasm as in the endosperm of the higher plants. This form of segmentation occurs also in *S. decipiens* and, according to my observation, is more common than that described by HARPER.

KUSANO saw and figured the clusters of small nuclei due to various sorts of amitosis as described in my former paper (5), but he did not study them carefully and failed to ascribe to them the importance that they really possess. He repeats the statement of his preliminary paper that the chromosomes number constantly five, and thus adds strongly to the case against the individuality of the chromosomes made in the present paper.

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EXPLANATION OF PLATES XVI–XVIII

All the figures were made with a 1.5$^{\mathrm{mm}}$ immersion objective and ocular 12 giving a magnification of 2130, excepting *fig. 36*, which was drawn under a 4$^{\mathrm{mm}}$ dry lens with a magnification of 400. They were reduced $\frac{1}{3}$ in reproduction, canceling the enlargement due to the camera and rendering them the same size as they were seen in the microscope.

PLATE XVI

FIG. 1.—A vegetative nucleus.

FIGS. 2–4.—Three stages in the formation of the spirem from the same cyst, showing also the asters of the preceding division in process of disintegration.

FIG. 5.—A large nucleus in spirem beside which is a small nucleus, derived by gemmation, still in the vegetative condition; from the same cyst as *figs. 6–9* and *13*.

FIGS. 6–9.—The development of the spindle, from the same cyst.

FIGS. 10–12.—The contraction of the spirem around the equator of the spindle; nuclear membrane with its chromatic granules dissolving; from the same cyst.

FIG. 13.—A young spindle with the chromatin so densely aggregated around the equator that different parts cannot be made out; from the same cyst as *figs. 5–9*.

FIG. 14.—A spindle at metaphase, showing three of the four chromosomes.

FIG. 15.—Chromosomes enlarging as they divide, each of them attached to the pole by heavy fibers.

FIG. 16.—A polar view of metaphase, showing the four chromosomes and the nucleolus.

FIG. 17.—Chromosomes divided and beginning to separate.

FIG. 18.—Anaphase, showing separating chromosomes and the heavy fibers which connect them across the equator of the spindle; from the same cyst as *figs. 15–17*.

FIG. 19.—Anaphase; chromosomes lost in the deeply staining mass at the poles; connecting fibers prominent.

PLATE XVII

FIG. 20.—Anaphase similar to *fig. 19*, but remarkable for the very late persistence of the aster from the previous division.

FIG. 21.—Similar anaphase, with the aster appearing unusually early.

FIG. 22.—Connecting fibers withdrawn.

FIG. 23.—Spindle breaking apart in the middle.

FIG. 24.—Radiations from the chromatic mass just beginning to appear at the lower pole; at the upper pole the focus has shifted out beyond the chromatin.

FIG. 25.—A stage in the development of the aster intermediate between the two poles of the spindle shown in *fig. 24*.

FIG. 26.—Aster strongly developed, while the chromatin remains extremely closely condensed; from the same cyst as *fig. 24*.

FIG. 27.—Chromatin beginning to enlarge to form karyosome.

FIG. 28.—Karyosome rounded off; nuclear vacuole just appearing.

FIG. 29.—Rays of aster beginning to bend around the vacuole to form nuclear membrane; nucleolus and remnant of spindle lying below daughter nucleus.

FIG. 30.—Rays of aster nearly surrounding the nuclear cavity; karyosome passing into spirem of the next mitosis; cf. *fig. 2*.

FIG. 31.—A vegetative nucleus with the old aster lying along side.

PLATE XVIII

FIG. 32.—Spindle of a primary nucleus with halo and disintegrating nucleolus.

FIG. 33.—Two sporangia from a newly segmented summer sorus, showing the character of the mitoses and the independence of the segments.

FIG. 34.—A spindle of one of the last mitoses of the sporangia.

FIG. 35.—*a*, group of spindles formed by the division of a cluster of nuclei formed by amitosis, which lies in the center of a large cyst, the periphery of which contains the spindles of many small nuclei (*b*) derived by gemmation.

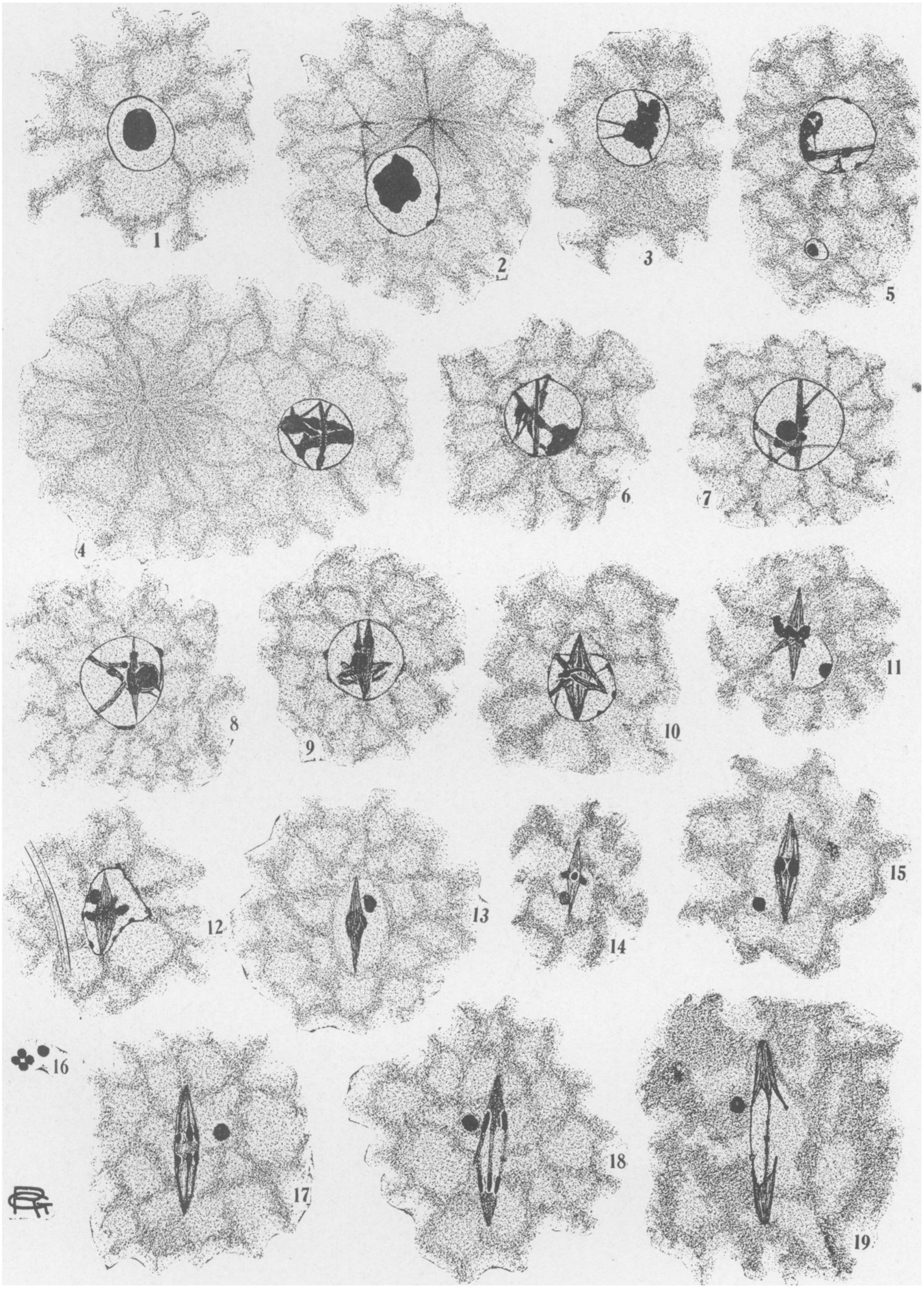
FIG. 36.—Amitosis by constriction in a primary cyst, showing very great differences in chromatin content of daughter nuclei.

FIG. 37.—An amitotic spirem in preparation for nuclear gemmation.

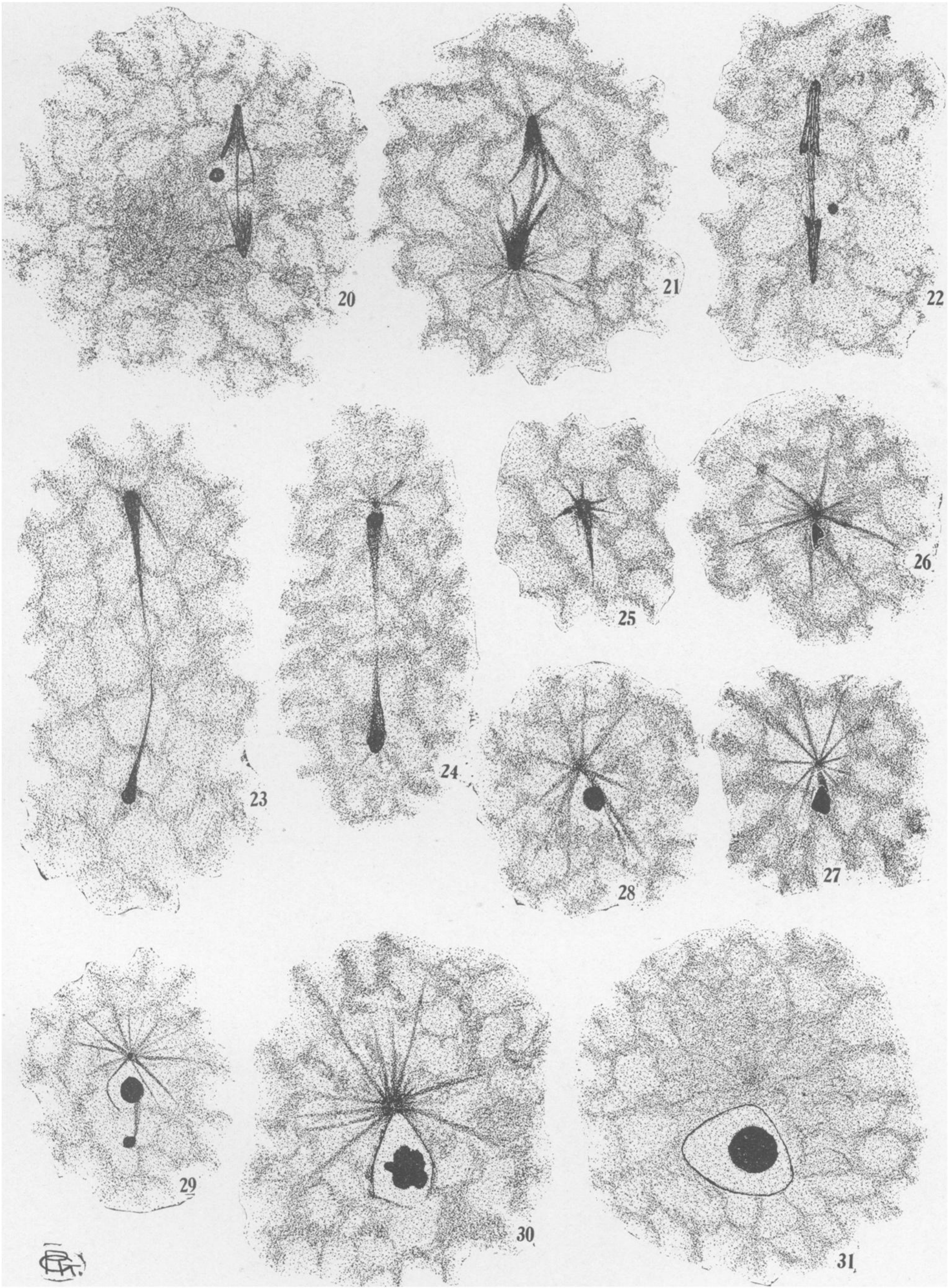
FIGS. 38–40.—Amitosis by constriction and by nuclear gemmation in the same cyst.

FIG. 41.—*a*, two amitotic nuclei; *b*, an adjacent nucleus in spirem, which though undoubtedly amitotic resembles mitotic spirems of *figs. 4, 6; c*, a cluster of amitotic nuclei from the same section.

FIG. 42.—A spindle with five chromatin bodies, presumably chromatin granules of the spirem not yet fused into chromosomes; cf. *fig. 11*.



GRIGGS on SYNCHYTRIUM



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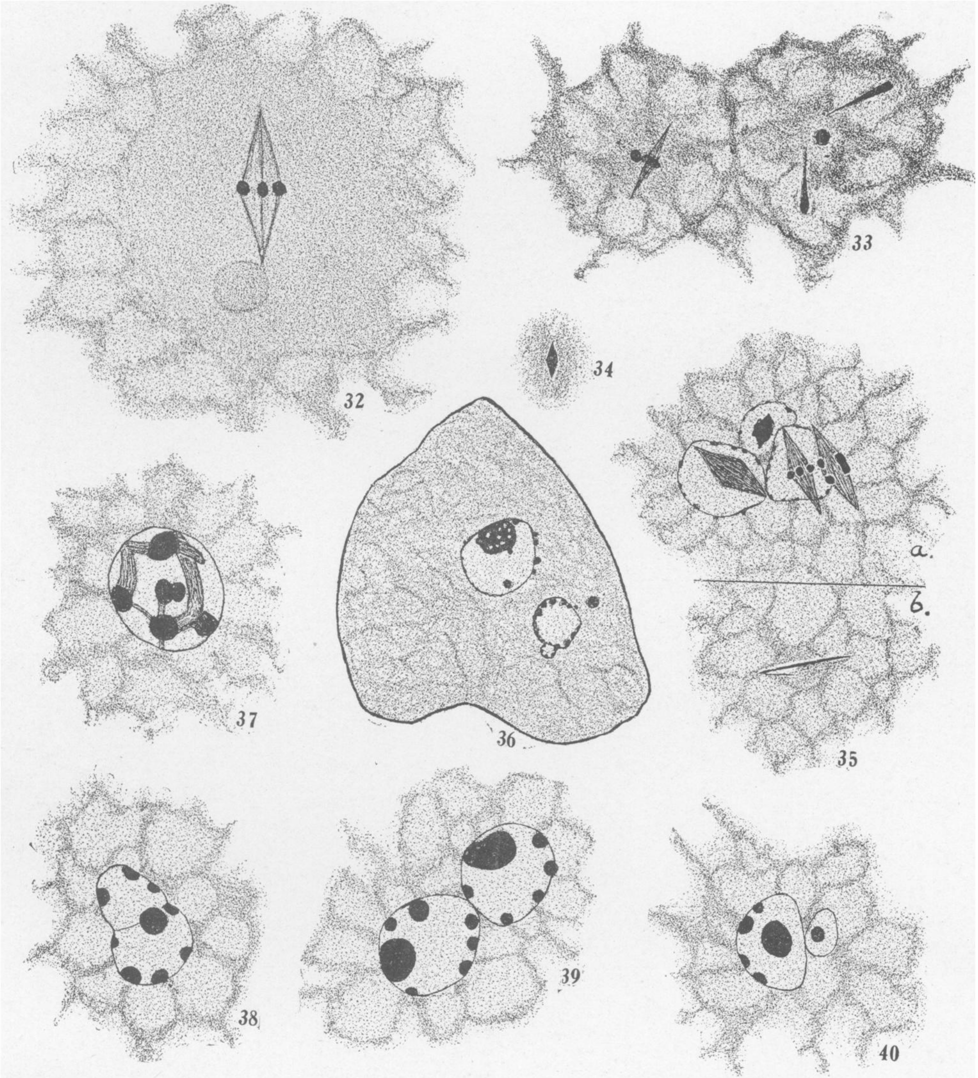

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